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RESEARCH ARTICLE

Development and Evaluation of a Plumbagin-Brinzolamide Binary Cocrystals for Improved Aqueous Solubility

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Abstract: Plumbagin (5-hydroxy-2-methyl-1,4-naphthoquinone) is a secondary metabolite which exhibit antioxidant, anti-inflammatory, and anticancer properties. Although therapeutically active, its clinical application is limited due to its poor aqueous solubility, which leads to suboptimal oral bioavailability and rapid systemic clearance. This research work aimed at the fabrication of a novel pharmaceutical cocrystal involving plumbagin and brinzolamide (PLB–BRZ) as an intervention to alter the physicochemical limitations of the parent phytoconstituent. The preparation of cocrystals involved three distinct approaches: neat grinding, liquid-assisted grinding, and solution crystallization across multiple stoichiometric ratios. Solid-state characterization of prepared cocrystals was carried out using Fourier Transform Infrared Spectroscopy (FTIR), Powder X-ray Diffraction (PXRD), Scanning Electron Microscopy (SEM), and Thermogravimetric Analysis (TGA) and the tests confirmed the formation of a distinct crystalline phase characterized by unique supramolecular synthons. Saturation solubility experiments in diverse media, including distilled water, 0.1 N HCl, and pH 6.8 phosphate buffer, revealed that the cocrystal prepared via solution crystallization (1:1 ratio) achieved a solubility increase exceeding 120% compared to pure plumbagin. These results show that the addition of plumbagin into a multicomponent crystalline lattice with brinzolamide effectively alters the hydration energy and lattice strength, providing a viable pathway for the development of high-performance botanical formulations. The results support the utility of supramolecular chemistry in overcoming the inherent delivery challenges associated with hydrophobic naphthoquinones.

Keywords: Plumbagin; Brinzolamide; Pharmaceutical Cocrystals; Solubility Modulation; Crystal Engineering.

1. Introduction

The identification of bioactive molecules from botanical sources provides a strong foundation for the development of therapeutic interventions targeting various pathological conditions. Among these, plumbagin, a naturally occurring yellow crystalline naphthoquinone, serves as a primary active constituent in the roots of *Plumbago zeylanica* L., a plant deeply rooted in traditional Chinese and Ayurvedic medicine [1]. This bioactive agent is prevalent within the Plumbaginaceae family and certain carnivorous plant genera, where it functions as a potent defense mechanism. Chemically, its structure as 5-hydroxy-2-methyl-1,4-naphthoquinone facilitates a wide range of biological interactions, resulting in documented antifungal, antiviral, neuroprotective, and anticancer activities [2].

The therapeutic efficacy of plumbagin is restricted by its solubility and poor dissolution rate as it belongs to Biopharmaceutics Classification System (BCS) Class II or IV candidate. These limitations culminate in low bioavailability and a short biological half-life, necessitating high or frequent dosing which often leads to radiomimetic and cytotoxic side effects [3]. Consequently, the development of advanced formulation strategies to improve the solubility and stability of this naphthoquinone is essential for its transition from laboratory research to clinical application.

Crystal engineering has gained prominence as a non-invasive method to alter the physical properties of active pharmaceutical ingredients (APIs) without modifying their intrinsic covalent structure. Pharmaceutical cocrystals are a class of multicomponent

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solids where the API and a stoichiometric amount of a co-former are held together by non-covalent interactions, such as hydrogen bonding and π- π stacking [4]. Unlike salts, cocrystals do not require ionizable functional groups, making them a versatile tool for neutral molecules like plumbagin. By carefully selecting a co-former that possesses complementary hydrogen-bond donor and acceptor sites, the lattice energy and thermodynamic stability of the crystal can be redirected to favor aqueous interaction [5].

In the current research work, brinzolamide was selected as a co-former due to its sulfonamide and secondary amine functionalities, which provide ideal sites for the formation of heterosynthons with the carbonyl and hydroxyl groups of plumbagin. This research evaluates the preparation and characterization of the PLB-BRZ cocrystal system, focusing on the impact of different crystallization techniques on the final solubility profile. The combination of these two entities into a single crystalline lattice aims to exploit supramolecular interactions to mitigate the hydrophobic nature of plumbagin [6].

2. Materials and Methods

2.1. Materials and Reagents

Plumbagin (PLB, 98% purity) was obtained from P.C. Chem, Mumbai, India. Brinzolamide (BRZ, 99.8% purity) was provided as a gift sample by Micro Labs Ltd., Bengaluru, India. Analytical grade ethanol and benzene were sourced from Loba Chemie Pvt. Ltd. All reagents were used as received without further purification. Distilled water was utilized for all solubility experiments

2.2. Preparation of PLB-BRZ Cocrystals

The development of the binary system involved three distinct mechanical and thermodynamic methods: Neat Grinding (NG), Liquid Assisted Grinding (LAG), and Solution Crystallization (SC) (Figure 2). Each method was evaluated at molar ratios of 1:1, 1:2, and 2:1 (PLB:BRZ) past literature on supramolecular synthesis [7]. The molar ratios are shown in Table 1.

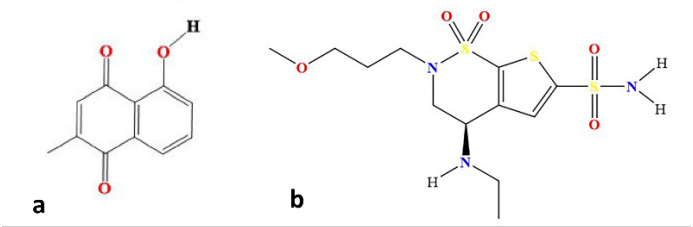

Figure 1. Structures of a. Plumbagin and b. Brinzolamide

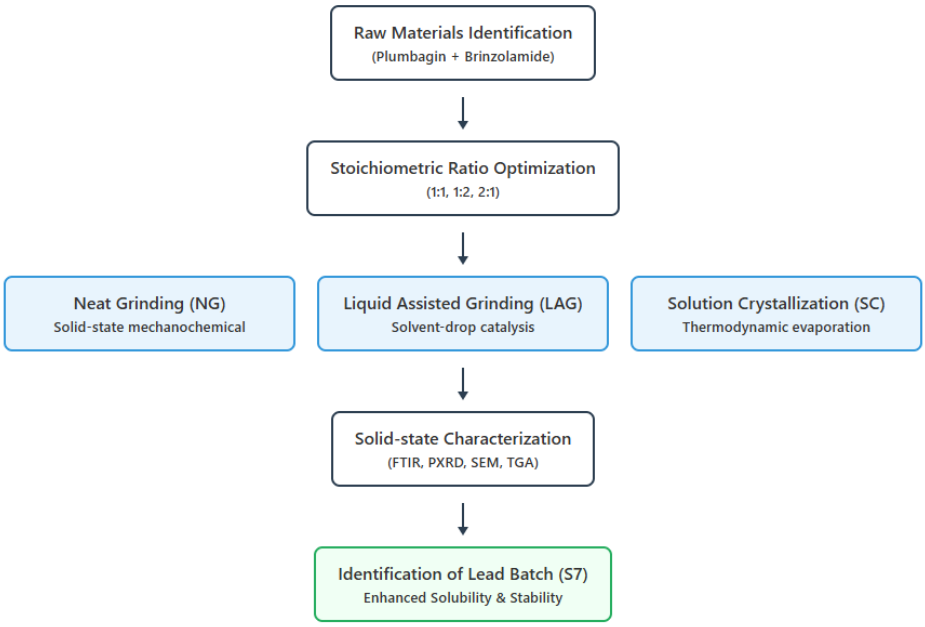

Figure 2. Experimental Methodology Involved in PLB–BRZ Cocrystals

2.2.1. Neat Grinding (NG) Method

Accurately weighed quantities of PLB and BRZ were transferred to a porcelain mortar. The mixture was subjected to intensive manual trituration for 30 minutes at room temperature. This solvent-free mechanochemical approach relies on the input of mechanical energy to facilitate molecular collision and subsequent rearrangement into a cocrystalline lattice [8]. The resulting homogeneous powder was collected and stored in a vacuum desiccator to prevent moisture absorption prior to analysis.

2.2.2. Liquid Assisted Grinding (LAG) Method

For the LAG process, the API and co-former were combined in the specified molar ratios and ground manually. During the 30-minute trituration period, a catalytic amount of ethanol (5–6 drops) was added periodically. The addition of a minor solvent phase acts as a molecular lubricant, enhancing the rate of cocrystal formation by increasing molecular mobility at the solid-solid interface [9]. The final product was dried at ambient temperature and stored in a desiccator.

2.2.3. Solution Crystallization (SC) Method

The SC method involved the dissolution of PLB and BRZ in a binary solvent system composed of ethanol and benzene (9:1 ratio). Initially, the components were ground with a few drops of ethanol for 30 minutes to ensure intimate contact. The mixture was then dissolved in the solvent blend and left for slow evaporation at room temperature. This thermodynamic approach allows for the gradual nucleation and growth of crystals from a supersaturated state [10]. The crystals formed upon complete evaporation were harvested and preserved under desiccation.

Table 1. Synthesis of BRZ-PABA cocrystals by three different methods.

S No.	Crystallization method	Stoichiometric ratio	Batch
1.	NG	1:1	N7
2.		1:2	N8
3.		2:1	N9
4.	LAG	1:1	L7
5.		1:2	L8
6.		2:1	L9
7.	SC	1:1	S7
8.		1:2	S8
9.		2:1	S9

2.3. Analytical Characterization

2.3.1. Equilibrium Solubility Studies

Saturation solubility was evaluated using the shake-flask method [11]. An excess of the samples was added to 10 ml of distilled water, 0.1 N HCl (pH 1.2), and phosphate buffer (pH 6.8). The suspensions were agitated at 20 rpm in a rotary shaker incubator maintained at 37 ± 0.5ºC for 48 hours to ensure equilibrium. After reaching equilibrium, the supernatants were filtered through 0.45 µm membrane filters. The concentration of PLB was determined using a double-beam UV-Vis spectrophotometer (Shimadzu UV-1800) at the maximum absorption wavelength (λ_{max}) of 419 nm [12].

2.3.2. Fourier Transform Infrared (FTIR) Spectroscopy

The chemical interactions and presence of supramolecular synthons between PLB and BRZ were investigated using a Shimadzu IRPrestige-21 spectrophotometer [13]. Samples were prepared using the KBr pellet technique, and spectra were recorded over the wavenumber range of 4000 to 400 cm^{-1} with a resolution of 4 cm^{-1}.

2.3.3. Powder X-ray Diffraction (PXRD)

The crystallinity and phase purity of the processed samples were assessed using a Malvern PANalytical Empyrean diffractometer, following standard diffraction protocols for pharmaceutical solids [14]. The instrument utilized Cu Kα-radiation (λ= 1.5406 Å) operating at 45 kV and 40 mA. Data collection was performed in the 2θ range of 5º to 35º.

2.3.4. Scanning Electron Microscopy (SEM)

Surface topography and crystal habit were visualized using a ZEISS Gemini scanning electron microscope. The samples were mounted on carbon tape and sputter-coated with gold to ensure conductivity and minimize charging effects [15]. Imaging was performed at various magnifications to observe the differences in particle shape and size distribution.

2.3.5. Thermal Analysis

Thermal stability and phase transitions were monitored using a PerkinElmer Pyris Diamond TG/DTA analyzer. Approximately 5 mg of the sample was placed in a platinum crucible and heated from 25ºC to 400ºC at a constant rate of 10ºC/min under a nitrogen atmosphere (150 ml/min) to determine weight loss profiles and decomposition temperatures [16].

2.4. Statistical analysis

Data are shown as mean ± standard deviation (n=3) for each experiment. Statistical analyses were conducted using GraphPad Prism software (version 5.04, GraphPad Software, USA)

3. Results

3.1. Design of Cocrystals of PLB

The successful synthesis of pharmaceutical cocrystals relies on the identification of supramolecular synthons structural units within a crystal formed through non-covalent interactions. Plumbagin (PLB) possesses a naphthoquinone core with two carbonyl oxygen atoms (C1=O and C4=O) and a phenolic hydroxyl group at the C5 position. The hydroxyl group serves as a hydrogen bond donor, while the carbonyl oxygens act as potent acceptors [14] (Figure 3). Brinzolamide (BRZ) is characterized by a secondary amine group and a primary sulfonamide moiety, providing multiple sites for hydrogen bonding.

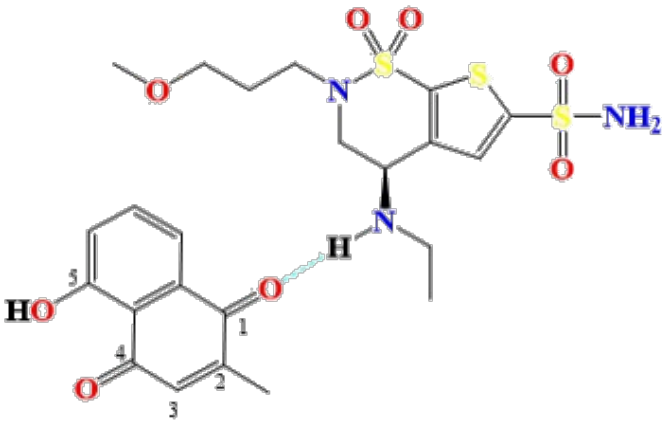

Figure 3. Probable Heterosynthon formation of PLB-PABA cocrystals

The design of the PLB-BRZ cocrystal was predicated on the formation of heterosynthons between the ketonic oxygen of PLB and the amino nitrogen of BRZ. Furthermore, the aromatic naphthoquinone ring of PLB and the thiophene ring of BRZ are likely to undergo π- π stacking interactions, which contribute to the stabilization of the multicomponent lattice. These interactions effectively mask the hydrophobic regions of the molecules, facilitating better interaction with aqueous solvent molecules during dissolution [15].

3.2. Saturation Solubility

Solubility enhancement is a primary objective in the development of pharmaceutical cocrystals. The saturation solubility of pure PLB and its synthesized cocrystals was evaluated across three different media to simulate various physiological environments (Figure 4). Pure PLB exhibited low solubility in distilled water (75.06 ± 2.18 µg/ml), 0.1 N HCl (154.06 ± 5.32 µg/ml), and pH 6.8 buffer (148.14 ± 2.79 µg/ml).

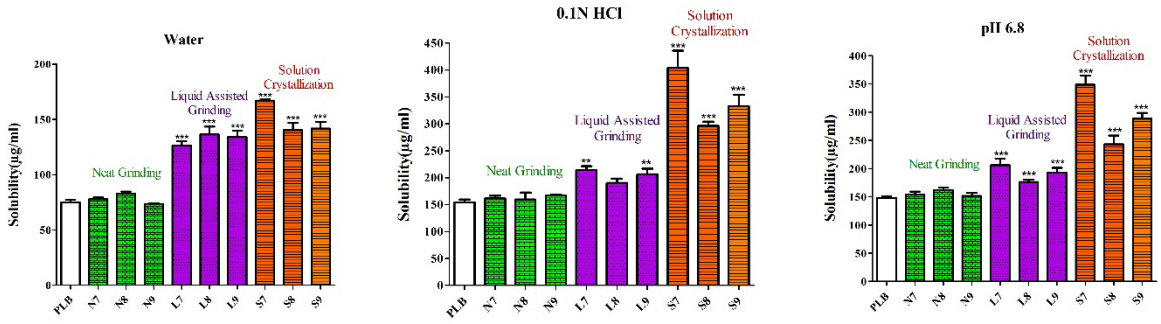

Figure 4. Saturation solubility of PLB and cocrystals in a) Distilled water, b) 0.1 N HCl, and c) pH 6.8 buffer.

The cocrystal prepared via solution crystallization in a 1:1 ratio (Batch S7) demonstrated the most significant improvement. Specifically, the S7 cocrystal achieved a solubility increase of approximately 122% in water, 135% in 0.1 N HCl, and 115% in pH 6.8 buffer compared to the parent API. This enhancement can be attributed to the reduction in lattice energy and the increased hydration of the new crystalline phase. The cocrystals synthesized via Liquid Assisted Grinding (LAG) also showed improved solubility, albeit to a lesser extent than the SC-derived products, suggesting that the SC method provides superior phase purity and more stable crystal growth [16].

3.3. FTIR Spectroscopy

FTIR spectroscopy was used to identify the functional groups involved in the supramolecular assembly. The spectrum (Figure 5) of pure PLB is characterized by sharp carbonyl stretching vibrations at 1649.63cm^{-1} (C1=O) and 1606.12 cm^{-1} (C4=O). Brinzolamide displays characteristic bands at 3313.62 cm^{-1} (N-H stretching) and 1342.39 cm^{-1} (S=O stretching).

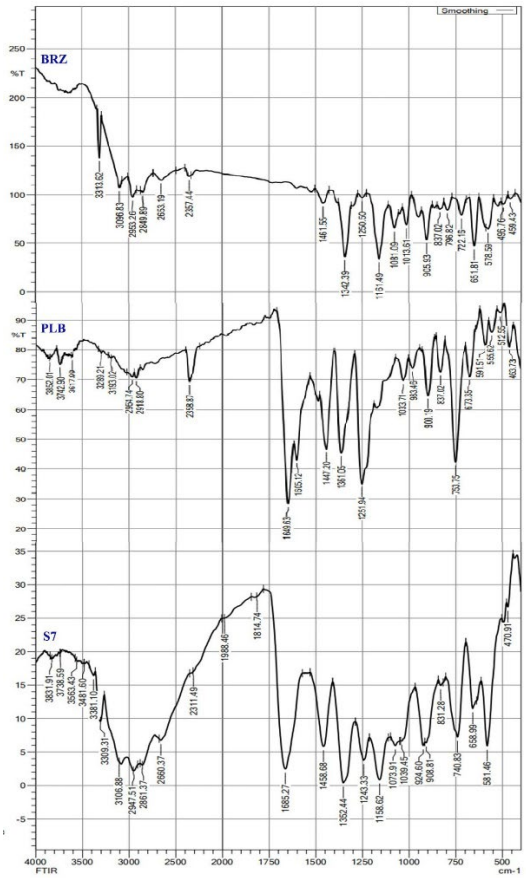

Figure 5. FTIR Spectra of PLB, BRZ and PLB-BRZ cocrystals(S7)

In the S7 cocrystal spectrum, a significant shift in the C1=O carbonyl peak from 1649.63 cm^{-1} to 1685.27cm^{-1} was observed. This "red shift" and the accompanying peak broadening indicate the formation of intermolecular hydrogen bonds between the carbonyl oxygen of PLB and the secondary amine or sulfonamide protons of BRZ [17]. The preservation of the primary peaks of both components with subtle shifts confirms the presence of both moieties in the cocrystal without covalent modification.

3.4. Phase Identification via PXRD

The PXRD patterns (Figure 6) provide conclusive evidence of the formation of a new crystalline phase. Pure PLB shows intense crystalline peaks at 2θ values of 11.22º, 13.05º, and 26.44 º. BRZ exhibits distinct reflections at 12.13º and 20.65º. The diffractogram of the S7 cocrystal (PLB-BRZ) reveals the emergence of entirely new peaks at $9.12^º, $12.50º, and $18.27^º, while many characteristic peaks of the individual components either disappeared or significantly shifted in intensity. This disappearance of parent peaks and the appearance of a new set of reflections is a hallmark of cocrystal formation, indicating the creation of a unique crystal lattice distinct from a physical mixture [18].

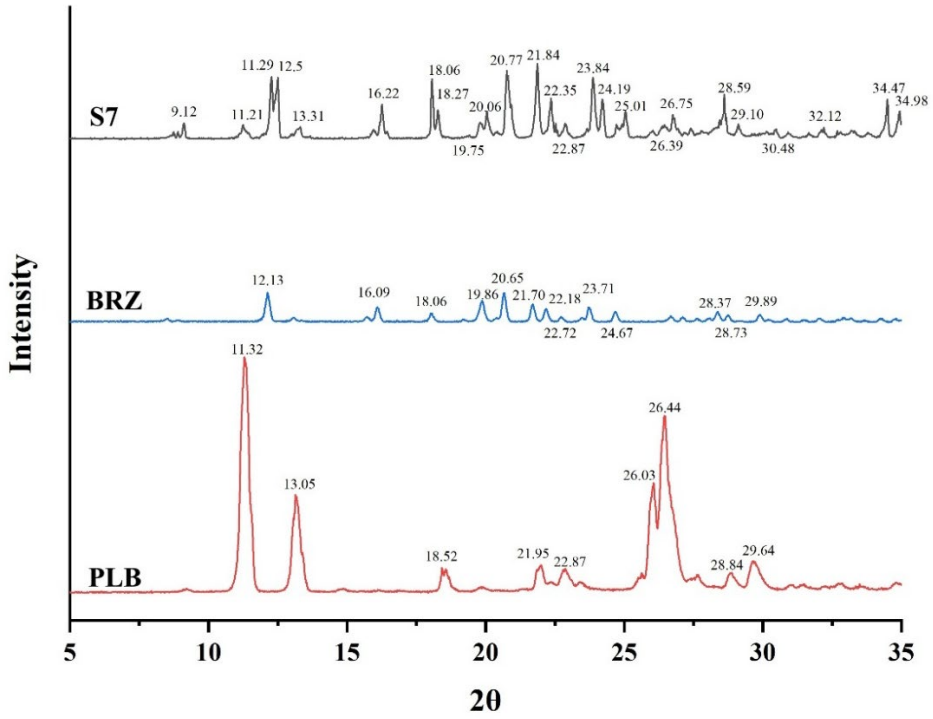

Figure 6. XRD patterns of PLB, BRZ and PLB-BRZ cocrystals(S7).

3.5. Morphological Characterization (SEM)

The SEM micrographs (Figure 7) revealed distinct changes in the crystal habit. Pure PLB appeared as irregular, needle-like crystalline particles, while BRZ was characterized by a globular, somewhat amorphous-like morphology. The S7 cocrystal displayed a markedly different habit, characterized by larger, flattened, and irregular crystalline plates. This change in morphology suggests a different growth mechanism and confirms the generation of a new solid phase. Such morphological changes can influence the surface area and dissolution rate, complementing the solubility findings [19].

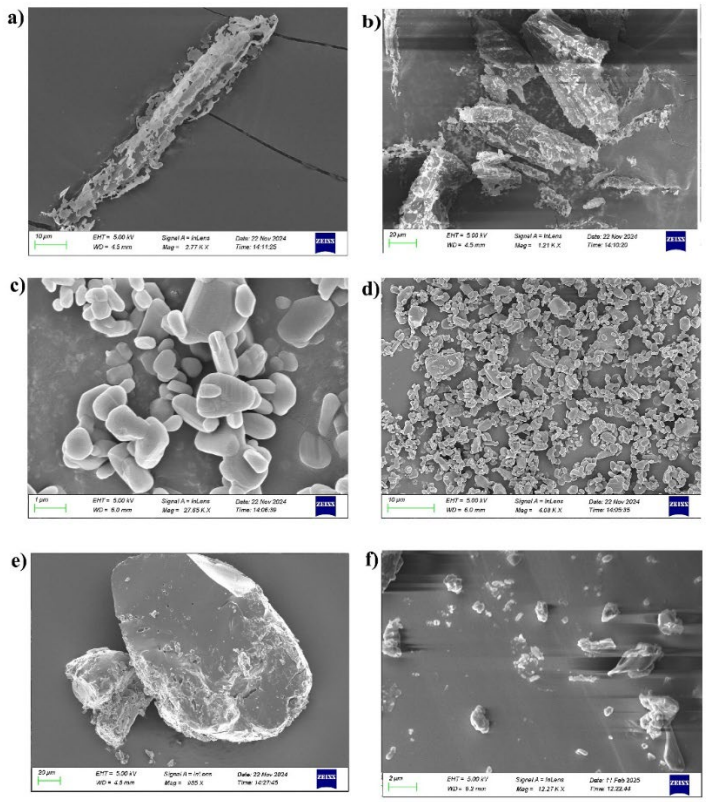

Figure 7. SEM Micrographs of a. PLB (2.77 KX), b. PLB (1.21 KX) c. BRZ (27.65 KX) d. BRZ (4.08 KX) e. S7 (985 X) and f. S7 (12.27 KX)

3.6. Thermal Stability and Phase Transitions (TGA)

Thermogravimetric analysis (TGA) was utilized to determine the thermal robustness and composition of the cocrystal. Pure PLB showed a rapid weight loss event (95.5%) starting at 110ºC with a T_{max} of 203.53ºC (Figure 8). BRZ exhibited a decomposition event beginning at 200 ºC with a T_{max} of 212.34ºC. The S7 cocrystal showed a modified weight loss profile (58.2% loss) occurring between 150ºC and 400ºC, with an increased T_{max} of 214.99ºC. The shift in decomposition temperature and the distinct weight loss percentage further validate the existence of a new crystalline entity with enhanced thermal stability compared to pure plumbagin [20].

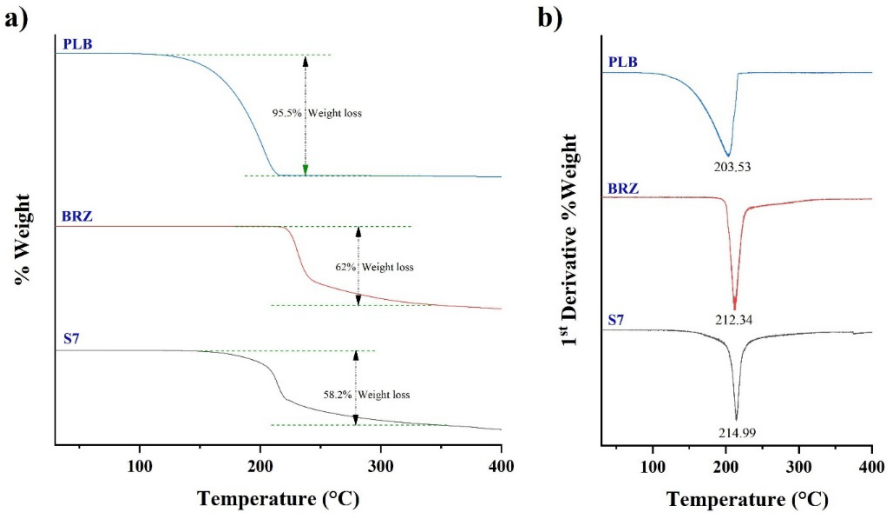

Figure 8. a. TGA of PABA, Plumbagin and PLB-PABA, b. DTG of PABA, Plumbagin and PLB-PABA

4. Conclusion

Pharmaceutical cocrystal of plumbagin were successfully developed using brinzolamide as a co-former through a variety of mechanochemical and thermodynamic techniques. The solution crystallization method at a 1:1 molar ratio yielded the most effective crystalline phase (Batch S7), characterized by significant enhancements in aqueous solubility across various pH conditions. Solid-state characterization through FTIR, PXRD, SEM, and TGA consistently verified the formation of a distinct binary system held together by supramolecular synthons. This crystal engineering approach effectively addresses the historical challenges associated with the poor solubility of plumbagin while potentially improving its therapeutic efficacy. The results suggest that the PLB-BRZ cocrystal is a promising candidate for further development into advanced drug delivery systems, offering a viable strategy for improving the biopharmaceutical performance of other hydrophobic phytoconstituents.

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